



Research Article

Conventional and Molecular Based studies on *Trichomonas vaginalis* in Quetta, Balochistan

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Abstract

This study was designed to investigate the prevailing status of *Trichomonas vaginalis*, a protozoan parasite causing urogenital tract infections in human beings, in Quetta, Balochistan, Pakistan. As no documented study of this kind has been conducted so far, it was designed to obtain the overall prevalence of *T.vaginalis* infection. Through conventional wet mount technique, its morphological identification by staining techniques and with PCR confirmation. In addition to investigation of age-wise and gender-wise prevalence of *T.v*, its correlation with reproductive disorders was also investigated through a questionnaire proforma while taking urine samples. For this purpose six hundred (600) urine samples (300 samples from each gender) were collected from the public sector hospitals located in Quetta city. The study was designed in three age groups as G1: 21-30, G2: 31-40, and G3: 41-50 years of each gender. A total of four samples were found positive microscopically only in female patients, whereas three of these positive samples were confirmed as *T. vaginalis* by PCR using BTUB9/2 set of primers. The fourth positive sample might be the specie other than *T.vaginalis*. It is concluded that the overall prevalence of *T.v* amongst females was observed as 0.1%, while the samples from males were found negative for *T.v*. so the overall percentage in total samples found as 0.5%.

Keywords: *Trichomonas Vaninalis*, urine sample, Microscopy, PCR, Prevalence.

ARTICLE INFORMATION

Received: 16.07.2019

Revised: 19.08.2019

Accepted: 30.09.2019

DOI: 10.31580/pjmls.v2i3.1112

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INTRODUCTION

Trichomonas vaginalis is a flagellated protozoan parasite, mostly exists as pyriform shaped but sometimes it acquires amoebic morphology. It lives extracellular in genitourinary track epithelium with primary anaerobic in existence [15]. *T.vaginalis* is distributed worldwide and it prevails in developed countries between women and men about 5% to 20% and 2%- 10%, respectively [11]. TV is ordinary non-viral sexually transmitted infection (STI) across the globe. World Health Organization (WHO) projected that there were 276.4 million cases reported in 2008 and about 90 % of disease took place in those whose livelihood means were in lesser quantity. This infection occurs higher than *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and syphilis. World wide it prevails 8.1 % in females and 1.0 % in males [29]. This study was designed to investigate the prevailing status of *Trichomonas vaginalis* in Quetta, Balochistan, Pakistan. We designed the study to investigate the overall prevalence of *T. vaginalis* infection through conventional wet mount technique, its morphological identification by staining techniques and by PCR confirmation. In addition to

investigation of age-wise and gender-wise prevalence of *T. vaginalis*, its correlation with reproductive disorders was also investigated.

Table. 1. PCR reaction mixture.

Reagents	Quantities used
Master mix	10 µl
BTUB9 _ _ F	1 µl
BTUB2 _ _ R	1 µl
DNA Template	3 µl
Molecular grade water	5 µl
Total volume of reaction	20 µl

A total 20 µl reaction mixture was taken to obtain the PCR product

Table. 2. Cycling conditions of PCR

Stages	Steps	Temperatures	Timing
Hold1	Initial Denaturation	95 °C	5 min
	Denaturation	95 °C	45 sec
	Annealing	58.1 °C	45 sec
45 cycles	Extension	72 °C	1 min
	Final Extension	72 °C	7 minutes

45 cycles were carried out on the mentioned temperatures



Table 3. Age wise prevalence of T.v in female patients

Age groups (years)	Total No of samples	No of positive samples	Prevalence percentage
21-30	100	1	1%
31-40	100	1	1%
41-50	100	1	1%
	300	3	1%

3 positive cases were observed in 300 female patients showing 1% prevalence

Table 4. Age wise prevalence of T.v in male patients

Age groups (years)	Total No of Samples	No of positive Samples	Prevalence percentage
21-30	100	0	0
31-40	100	0	0
41-50	100	0	0
	300	Nil	Nil

No positive case was observed during the screening of 300 male patients

there is no documented study of the same kind conducted in this study area.

Age-wise Prevalence

The rate of prevalence was studied in different age groups in the study population. For this purpose three age groups were selected as G1: 21-30 years of age, G2: 31-40 years of age and G3: 41-50 years of age.

Gender-wise Prevalence

The rate of prevalence of *T. vaginalis* was studied both in female and male patients in the study population. For this purpose urine samples from male and female patients were compared and data was recorded.

Molecular Techniques for identification of *T. vaginalis* from urine samples

DNA Extraction

Microscopically confirmed samples were subjected to DNA extraction and PCR. For this purpose urine samples were centrifuged and the sediments containing the organism were used for DNA extraction. The Boiling technique was used for DNA extraction [19] [25].

PCR

BTUB 9/2 which are 100% species specific oligonucleotide primers and 100% sensitive with 112 bp were designed [8]. Conventional PCR was performed to amplify genes of interest, as follows;

The reaction was performed in a total volume of 20 μ l reaction mixture. PCR reaction mixtures and the PCR cycling conditions tabulated in table 1 and 2.

Gel Electrophoresis

1. Five μ l of obtained product was confirmed through 2% Gel electrophoresis
2. The PCR product was electrophoresed and later visualized in UV illuminator to observe the amplified product [25].

Statistical Analyses

The data was analyzed by descriptive statistics as percentage prevalence of the study.

RESULTS

Collection of samples: study area, sample size and study design.

For this study we selected the public sector hospitals in Quetta city of Balochistan. We went through 600 patients and processed urine samples from different individuals randomly. The samples were analyzed by conventional microscopic and molecular techniques.

Conventional techniques for isolation and identification of *T. vaginalis* from urine samples.

A total of six hundred (600) fresh urine samples were taken, processed and examined microscopically by wet mount technique for motility and Giemsa's staining for species

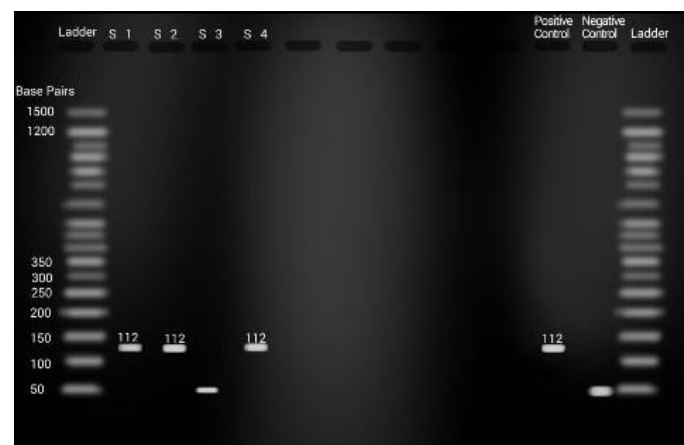


Fig. 1. PCR product 112 bp using BTUB9/2 primer to amplify specific sequence of genomic DNA of *T.v*

METHODOLOGY OF THE STUDY

Collection of samples: study area, sample size and study design.

In the current study a total of 600 urine samples (300 samples each gender) were collected randomly from patients visiting public sector hospitals at Quetta, Balochistan. While attending gynecological OPD, female patients were investigated for relative gynecological problems, relative sign symptoms and took urine samples (300 Samples). Moreover, urine samples of male patients (300 samples) were also analyzed for TV, received in the Microbiology/Pathology laboratories of the public sector hospitals for routine microscopy of urology.

All informative data regarding age, gender, history and types of reproductive disorders in females were collected on a questionnaire prepared for the said purpose.

Conventional techniques for isolation and identification of *T. vaginalis* from urine samples.

Fresh urine samples taken were processed and examined microscopically by wet mount technique for motility and staining for specie identification [16, 28, 30].

Epidemiological Parameters

Overall Prevalence

The overall prevalence of *T. vaginalis* was estimated in the studied population as a base line study because up to date



identification. The prevalence of *T. vaginalis* was recorded. The duration of the study was almost three months period from May 4th July to 29th 2019.

Epidemiological Parameters

Age wise and Gender wise prevalence

The age groups amongst male and females were designed as follows Table 3 and 4.

In the current study three samples were positive microscopically amongst 300 females. The rest of 300 male patients samples were negative microscopically. The percentages were calculated accordingly (Table 3 and 4).

In female, the first age group designated was 21-30 years of age with a total of 100 patients samples amongst whom one sample was positive with a percentage of 1% whereas in group second designated as 31-40 years of age contained 100 patients with 1 positive case and the percentage was found as 1% and in the third age group designated as 41-50 years of age contained 100 patients in which 1 case was recorded to be positive and the percentage observed was 1%. All positive cases lied between the ages of 25, 30, 31, 45 years with relevant notable signs & symptoms of reproductive disorders, including burning and itching.

The overall frequency amongst females was observed as 1% while that of males triggered during the study was noticed as zero, so the overall percentage in total samples was found as 0.5%.

Molecular Techniques for identification of *T. vaginalis* from urine samples

DNA Extraction

Microscopically confirmed samples were subjected to DNA extraction and PCR. Positive urine samples were centrifuged and the sediments containing the organism were used for DNA extraction. Rapid boiling technique was used to collect DNA for PCR analysis [19, 25].

PCR

BTUB9/2 gene was targeted to capture the parasitic DNA amplification that showed the following results. Out of four positive samples three were found positive for *Trichomonas vaginalis* where as one did not matched the desired bp size.

Gel Electrophoresis

DNA obtained was confirmed through gel electrophoresis and further the obtained DNA was amplified via conventional PCR. The PCR product was electrophoresed and later visualized in UV illuminator to observe the amplified product.

DISCUSSIONS

Numerous studies have been conducted by many researchers on *Trichomonas vaginalis* around the globe to study its prevalence, genotypic and phenotypic properties. It is a known STD pathogen around the world and is a major cause of human reproductive disorders. Nevertheless, this parasite is present everywhere, and has been isolated from vaginal secretions and urine from humans.

Urine samples were used in the study for the detection of *Trichomonas vaginalis* in both males and females (300 each) as to know the frequency of occurrence in both genders. The study was random, non symptom based and no group relating

ethnic, class or society was targeted to observe the occurrence taking in mind the reality that in our society no one admits to volunteer for STD related research. Thus urine was decided to be ideal for detection of this particular pathogen.

We collected 600 samples out of which three (3) were found positive in females while males were found negative, comparing it with a study in Papua New Guinea [7] showed that the male indigenous population had a prevalence of 10.9% compared with a prevalence of 0% in the male white population.

Open usage of self medication like azoles and antibiotics does affect the presence of parasite as during the conduction of study we came across patients who were using the medications that may affected the parasite and may have lowered the prevalence rate. Moreover, we also focused on the frequency of urination at the time of sample collection which also had affect on the prevalence rate of parasite, as the prevalence rate could be high at first urination.

Wet mount microscopic technique was used for the detection of parasite in urine samples as per steps [16] and was observed under 10 x and 40 x within 10 min of collection [28]. Geimsas staining was done as applied by [30] and purple stained organisms were observed under microscope. The same staining technique was also applied earlier [16].

For molecular studies DNA was isolated through rapid boiling technique [19] [25] and the obtained product was observed via gel electrophoresis. BTUB9/2 primers were used to identify the desired species which detected three out of four to be as *Trichomonas vaginalis*, as already used [25] and conventional PCR was carried out to obtain results [25].

Gel electrophoresis was performed by making 2% agarose gel for the detection of results under uv illuminator. The desired base pair sequence was 112bp which was obtained as per procedure [25].

REFERENCES

1. Awwiuro OG, Diagnosis of trichomoniasis in pap smears; How effective is it? European Journal of Experimental Biology.2011; 1 (1): 10-13.
2. Caliendo AM, Jordan JA, Green AM, Ingersoll J, Diclemente RJ, Wingood GM, Real-time PCR improves detection of *Trichomonas vaginalis* infection compared with culture using self-collected vaginal swabs. Infectious Disiseases in Obstetrics and Gynecology. 2005; 13(3):145–150.
3. Camila BM, Mariana DSM, Tiana T, Comparison of permanent staining methods for the laboratory diagnosis of Trichomoniasis. Rev. Inst. Med. Trop Sao Paulo. 2015; 58: 5-9.
4. Donald ER, Marlilyn CR, Tom T, John NK, Development of a Polymerase Chain Reaction-Based diagnosis of *Trichomonas vaginalis*. Journal of Clinical Microbiology. 1992; 30: 465-472.
5. Danesh IS, Stephen JM, Gorbach J, Neonatal *Trichomonas vaginalis* infection. J. Emerg. Med. 1995; 13: 51–54.
6. Jackson DJ, Rakwar JP, Bwayo JJ, Kriess JK, Moses S, Urethral *Trichomonas vaginalis* infection and HIV-1 transmission. Lancet. 1997; 350(9084):1076.
7. Francis JB, Geoffrey PG, *Trichomonas vaginalis* epidemiology: parameterising and analysing a model of treatment interventions. Sex Transm Inf. 2000; 76: 248–256.
8. Guillermo M, Thomas CQ, Annerompalo, Kelly TMJR, Charlotte AG, Diagnosis of *Trichomonas vaginalis* infection by PCR using vaginal swab samples. Journal of Clinical Microbiology. 1998; 36: 3205–3210.
9. George PS, Linda CM, Akbar AZ, Stephan JK, Evaluation of six media for the growth of *Trichomonas vaginalis* from vaginal secretions. Journal of Clinical Microbiology. 1989; 27 (6): 1230-1233.



10. Heine P, McGregor JA, *Trichomonas vaginalis*: a reemerging pathogen. Clin. Obstet. Gynecol. 1993; 36:137–144.
11. Ioanna KH, Carageorgiou, D. Varonos, S. Tzannetis, Growth and survival of *Trichomonas vaginalis*. J. Med. Microbiology. 1998; 47: 555-560.
12. Jane RS, Lisa FL, Improved detection by DNA amplification of *Trichomonas vaginalis* in males. Journal of Clinical Microbiology. 2002; 40(10): 3681–3683.
13. Krashin JW, Koumans EH, et al., Jamie WK, Emilia HK, Ayanna CBS, Jim RB, W. Evan S, Mary KS, Lauri EM, *Trichomonas vaginalis* prevalence, incidence, risk factors and antibiotic-resistance in an adolescent population. Sexually Transmitted Diseases. 2010; 37(7): 440-444.
14. Klinger EV, Kapiga SH, Sam NE, Aboud S, Chen CY, Ballard RC, Larsen U, A community-based study of risk factors for *Trichomonas vaginalis* infection among women and their male partners in Moshi urban district, Northern Tanzania. Sexually transmitted diseases. 2006; 33(12): 712–718.
15. Lisa FL, Spencer RH, Jane RS, Detection of Trichomonosis in vaginal and urine specimens from women by culture and PCR. Journal of Clinical Microbiology. 2000; 38 (10): 3585–3588.
16. Mohammad YA, S. Sivanandam, G. Suresh K, Modified field's staining; a rapid stain for *T. vaginalis*. Diagnostic Microbiology and infectious diseases. 2010; 68:159-162.
17. Mohsin SK, Magnus U, Shakila Z, Cecilia SL, HIV, STI prevalence and risk behaviours among women selling sex in Lahore, Pakistan. BMC Infectious Diseases. 2011; 11:119-127.
18. Madeline S, Maya S, Emilia HK, Geraldine M, Stuart B, Lauri M, The prevalence of *Trichomonas vaginalis* infection among reproductive-age women in the United States, 2001–2004. Clinical infectious diseases. 2007; 45:1319–26.
19. Men-Fangs S, Pey-Rulin L, JAH-YAO L, Colorimetric one-tube nested PCR for detection of *Trichomonas vaginalis* in vaginal discharge. Journal of clinical microbiol. 1997; 35(1): 132-138.
20. Patrica K, *Trichomonas vaginalis*: a review of epidemiologic, clinical and treatment issues. Kissinger BMC Infectious Diseases. 2015; 15:307-314.
21. Prashini M, David W, Cathy C, Jack M, A. Willem S, *Trichomonas vaginalis* is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus. Clinical Infectious Diseases. 2002; 34: 519–22.
22. Ragaa M I, Maisa A S, Diagnosis of *Trichomonas vaginalis* infection by PCR. Iranian Journal of Clinical Infectious Diseases. 2006;1(4):171-175.
23. Razia K, Noor J, Siraj A, Haris MK, Tamkin R, Comparison of four diagnostic techniques for detection of *Trichomonas vaginalis* infection in females attending tertiary care hospital of North India. Indian J. of Pathology and Microbiology. 2015; 58(1): 36-39.
24. R. Scott M, Laura S, Wisal MH, Ludo L, Kishorchandra M, James K, Jeckoniah NA, Walter J, Jared M B, Infection with *Trichomonas vaginalis* increases the risk of HIV-1 acquisition. The journal of infectious diseases. 2007; 195: 698–702
25. Saleem KA, Sami RA, Haithem MHA, Identification study for suspected women with *Trichomonas vaginalis* by PCR technique in AL-Najaf AL-Ashraf province. Al-Kufa University Journal for Biology. 2018; 10 (1): 183-192.
26. Sheila N, Othman K, Stefanie S, Alfred O, Henry O, Joshua K, Irene N, Freddie B, Very low sensitivity of wet mount microscopy compared to PCR against culture in the diagnosis of vaginal trichomoniasis in Uganda: A cross sectional study. BMC res. Notes. 2017; 10: 259-265.
27. Tania C, Vicky J, Chanda M, Shepherd K, Judith V, Anne B, Non-sexual transmission of *Trichomonas vaginalis* in adolescent girls attending school in Ndola, Zambia. PLoS ONE. 2011; 6(1): 1-5.
28. Vartika P, Arti J, Avinash L, Kaore N, Tukaram P, Comparison of wet mount examination with Giemsa staining and fluorescent staining for detection of *Trichomonas vaginalis* in clinically suspected cases of vulvo-vaginitis. Int. J. Curr. Microbiol. App. 2017; 6 (3): 718-724.
29. World Health Organization (WHO) 2001. Global prevalence and incidence of selected curable sexually transmitted infections: overviews and estimates. In: WHO/ HIV/AIDS. Edited by Organization WH. Geneva.
30. Zhao-Rong L, Alvin AG, A simple and rapid method for staining *Trichomonas foetus* and *Trichomonas vaginalis*. J. Vet. Diagn. Invest. 1999; 11:471–474.
31. Z Valadkhani, F Kazemi, M Assmar, A Amirkhani, B Esfandehi, M Lotfi, S Ghobadirad, N Hassan, Z Aghighi, Molecular diagnosis of Trichomoniasis in negative samples examined by direct smear and culture. Iranian J Parasitol. 2010; 5(4): 31-36.

